Journal of Chromatography, 225 (1981) 196—201
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 931

Note

Gas chromatographic quantitation of two plasticizers contaminating intravenous fluids stored in plastic containers

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(Received March 2nd, 1981)

The plasticizer di(2-ethylhexyl) phthalate (DEHP) has been reported as a contaminant in biological fluids by several investigators. Ono et al. [1] detected measurable amounts of DEHP in peripheral blood samples of hemodialysed patients immediately after dialysis. Hillman et al. [2] measured the same plasticizer in autopsied tissue (heart and small intestine) of infants who had umbilical catheters inserted and received varying amounts of blood products. Plasticizer contamination has been reported by Roll et al. [3] in plasma and by Ishida et al. [4] during lipid analysis. The toxicity of plasticizers has also been the subject of several investigations. Calley et al. [5] have reported the LD_{50} of plasticizers in mice and also noted that DEHP shortened hexabarbital sleeping time. Aronson et al. [6] reported that DEHP significantly decreased spontaneous heart rate, coronary flow and isometric tension but elevated diastolic tension in isolated perfused heart. They also noted significant concentration changes in tissue glycogen, ATP, creatine phosphate, etc. Our toxicity studies [7] with DEHP and di-*n*-butyl phthalate (DBP) with dogs showed weight losses, incomplete excretion (especially of DBP), etc.

In addition to DEHP we have reported and quantified [by gas chromatography (GC)] and confirmed [by gas chromatography—mass spectrometry (GC—MS)] the presence of another plasticizer, DBP, in a very significant proportion (80%) of selected surgical patients [8]. Hence, in the light of our toxicity studies with plasticizers and those of other investigators, it was very important for us to determine the source of contamination. These studies were

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0378-4347/81/0000—0000/$02.50

in turn intended to reduce plasticizer contamination in our surgical patients, especially those with impaired renal function due to incomplete excretion of the plasticizers [8]. We report here our investigations of several possible sources of plasticizer contamination.

EXPERIMENTAL

Units of whole blood and packed red cells, stored in plastic CPD solution bags and deemed unfit for human use, were secured from the hospital blood bank. Random samples of 5% glucose in water and 0.9% sodium chloride; 0.9% sodium chloride solution and lactated Ringer's solution stored in the original commercial plastic bags were obtained for study from the surgical intensive care unit's supply closet on two random days apart (4 units each). The fluids (2-ml aliquots of blood or 100 ml of crystalloid solution) were extracted with 10 ml of Dole's solution [9] [2-propanol—*n*-heptane—sulfuric acid (40:10:1)]. The homogenate was centrifuged for 10 min at 4°C, the supernatant was diluted with water (20 ml) and *n*-heptane (10 ml), the solution was agitated and the phases were allowed to separate in a separating funnel. The *n*-heptane phase was dried over anhydrous sodium sulfate, evaporated to dryness under a nitrogen stream and the residue was dissolved in 500 μl of acetone for GC analysis (4 μl). Random samples of alcohol sponges used by our phlebotomy team and the rubber stopper of the blood collecting tubes were extracted with 10 ml of Dole's solution and worked up as above.

A blank prepared using doubly distilled water was run simultaneously through the entire chemical extraction process. The same source of distilled water was used to mix the reagents. Glassware was washed in an ultrasonic washer, rinsed with prechromatographed acetone and baked in an oven overnight at 200°C.

Gas chromatographic analysis

A Hewlett-Packard 5831A gas chromatograph, equipped with a dual flame ionization detector, automatic injector, data system and a 1.8 m × 2 mm I.D. glass column with a 10% Silar-10C packing (Applied Science Labs., State College, PA, U.S.A.) was used. The carrier gas was nitrogen at a flow-rate of 30 ml/min. The column was programmed from 145 to 225°C at 2.5°C/min [8]. The injector and detector temperatures were 300°C. The plasticizers were identified on the basis of their retention times compared with those of standards (Applied Science Labs.).

RESULTS

The levels of the plasticizers detected in various solutions are presented in Table I and the following were the sources of contamination:

(a) The highest levels of DEHP were measured in the blood components stored in CPD solution and plastic bags. The levels increased with the duration of storage, ranging from 3500 to 7500 μg per 100 ml soon after the 3 weeks' expiration date (Figs. 1 and 2C). There did not appear to be any significant difference in the DEHP levels of packed red cells and whole blood. No DBP was

TABLE I

LEVELS OF PLASTICIZERS FOUND IN VARIOUS INTRAVENOUS FLUIDS AND OTHER COMMONLY USED HOSPITAL MATERIALS

System	Concentration (μg per 100 ml)	
	DBP	DEHP
Ringer's lactate	Trace	0
0.9% NaCl	1.98, 3.6	1.17, 1.30
5% glucose—0.9% NaCl	2.25, trace	0.85, 1.25
5% glucose—water	2.33, 3.5	0.95, 0.90
Blood*	—	3500—7500
Alcohol sponges	1.2, 1.30, 1.8, 1.80, 1.90, 2.85 (each sponge)	—
Rubber stopper**	—	—

*See Fig. 2 for details.
**Contained a significant amount of another plasticizer (Fig. 3).

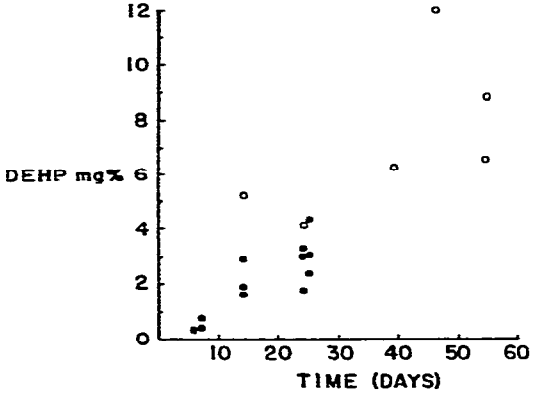

Fig. 1. Levels of DEHP measured in CPD blood stored in plastic bags. The levels of DEHP increased with the duration of storage. ○, whole blood; ●, packed cells.

detected in the above solutions.

(b) Both DEHP and DBP were measured in 0.9% sodium chloride solution, 5% glucose—0.9% sodium chloride solution and 5% glucose—water but only in ng/100 ml levels (Fig. 2B).

(c) Six alcohol sponges averaged 1.31 μg of DBP per sponge. No DEHP was detected.

(d) The red rubber stopper did not contain any DEHP or DBP. However, significant levels of another plasticizer were detected, but no attempt was made to identify it (Fig. 3).

DISCUSSION

Many plastic medical devices are possible sources of contamination with plasticizers of hospitalized patients. The devices involved in the administration of parental therapy that we have tested to date in our laboratory are significant

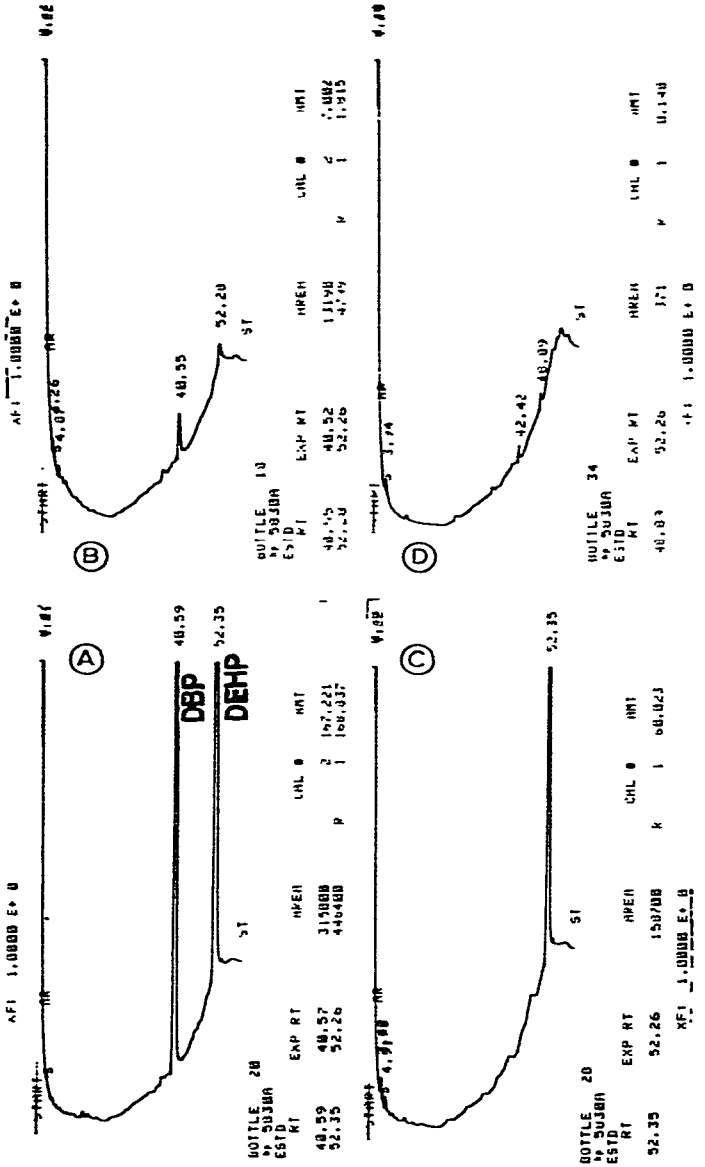

Fig. 2. Gas chromatograms of (A) DBP and DEHP standard solutions; (B) the lipid fraction of 5% G/W solution; (C) CPD stored blood; (D) distilled water blank. No DEHP or DBP were identified in the center blank (D). The highest level of DEHP was noted in the blood sample (C). Both DBP (40.55 min) and DEHP (52.28 min) were present in 5% glucose—water solution (B).

because any possible contamination will directly contaminate patients. Although other investigators [1—6] have measured the most commonly used plasticizer, DEHP, in patients and tissues, we have detected a second plasticizer, DBP, in our patients [8] and some of our crystalloids.

Our findings on DEHP contamination are in agreement with those of several other studies. Marcel and Noel [10] noted plasticizer contamination of blood

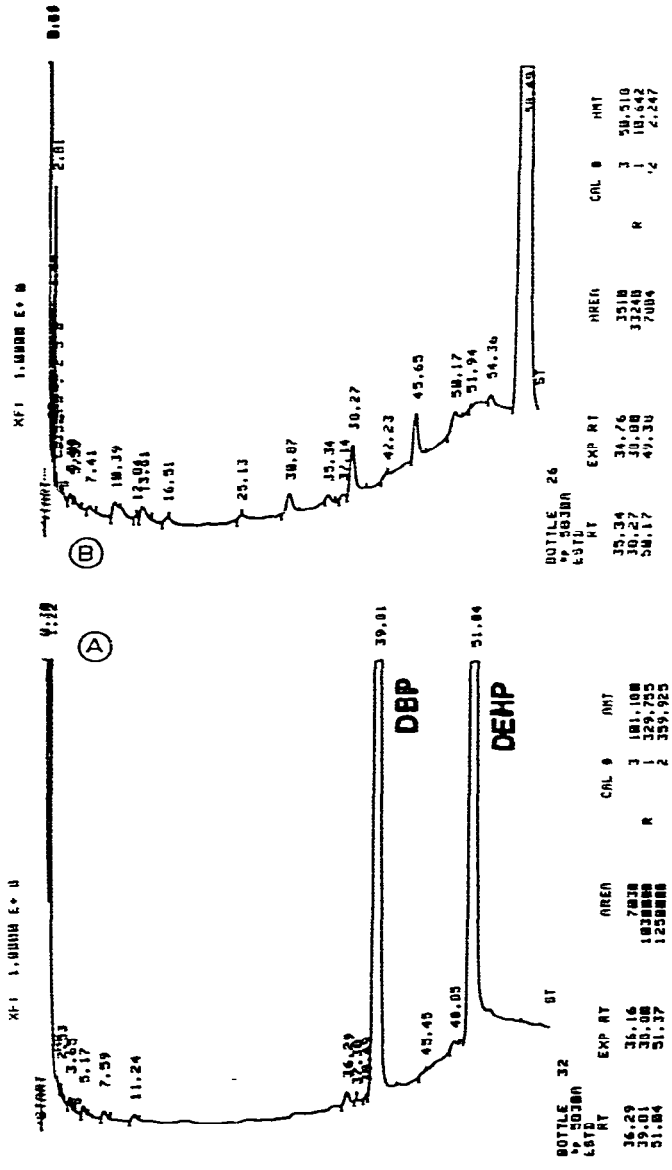

Fig. 3. Extractable lipid contents of the rubber stopper of blood collecting tubes. Composite chromatograms of (A) a standard solution of DBP and DEHP and (B) the lipid fraction extracted from a rubber stopper of a blood collecting tube. The major plasticizer, with a retention time of 58.49 min, extracted from the rubber stopper is well separated and distinguishable from DBP (39.81 min) and DEHP (51.04 min) in the standard solution. The small unidentified peak at 38.27 min can be separated by our column from DBP at 39.81 min into two separate peaks.

stored in plastic bags. Jaeger and Rubin [11, 12] detected measurable levels of DEHP in blood stored in standard plastic bags. The levels of extracted plasticizer increased with duration of storage and could be found in both the red

blood cell (10%) and plasma fractions (90%). Rossel and Bogaert [13] discussed the contamination of biological samples with tygon tubing, cork, plastic syringes, etc. Rock et al. [14] noted an increased concentration of DEHP during storage of whole blood, platelet-rich plasma and platelet concentrates.

The plasticizer DBP is recognized by several investigators as being more toxic than DEHP [5]. Our animal studies also revealed a greater toxicity from equal weight dosages of DBP and DEHP infusion [7]. Stetson and Autian [15] have extensively reviewed various plasticizer toxicity studies and believe that only a low order of acute systemic toxicity has been demonstrated in animal and human experiments. However, attention was first drawn to possible toxicity when cell cultures were destroyed when early plastic containers were used. Protein solution, e.g., blood, can extract more plasticizers from the containers. The addition of fat-soluble vitamins can also extract more of the plasticizers. We do not know the extent to which other medications added to intravenous solutions can increase the extracted plasticizer.

At present these devices have FDA approval. Except for the levels we have measured in stored blood the degree of contamination is small per device unit. However, if we take into account the total number of plastic devices, the extent of contamination could become significant. More studies on the actual contaminations and long-term toxic effects are required.

ACKNOWLEDGEMENTS

This work was supported in part by USPHS grants RR502, grant No. 19632-01 CCG and the John F. Linsley Fund.

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